



UNIVERSITY OF  
SAN FRANCISCO

# Determining the Impact of Double-Layer Formation on Electrostatic DNA Melting

Gayatri Raghu and Ryan M. West

University of San Francisco, Department of Chemistry, San Francisco, CA 94117

## Introduction

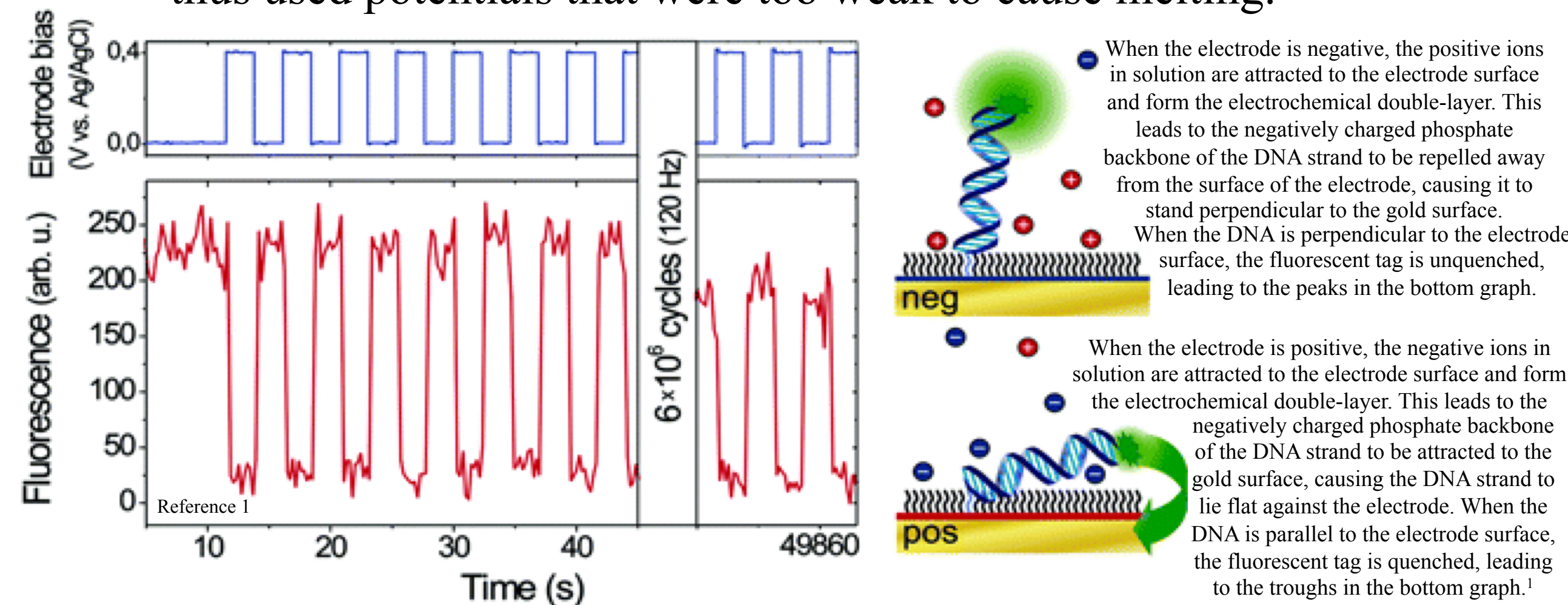
Electrostatic DNA melting can be utilized to detect the presence of mismatches in the DNA double helix. In this work, we investigate the mechanism of electrostatic melting. By monitoring the rate of melting while applying fast potential pulses, we can identify the extent to which the mechanism of electrostatic DNA melting is caused by the generation of the electric field within the electrochemical double-layer.

## Electrostatic Mechanism

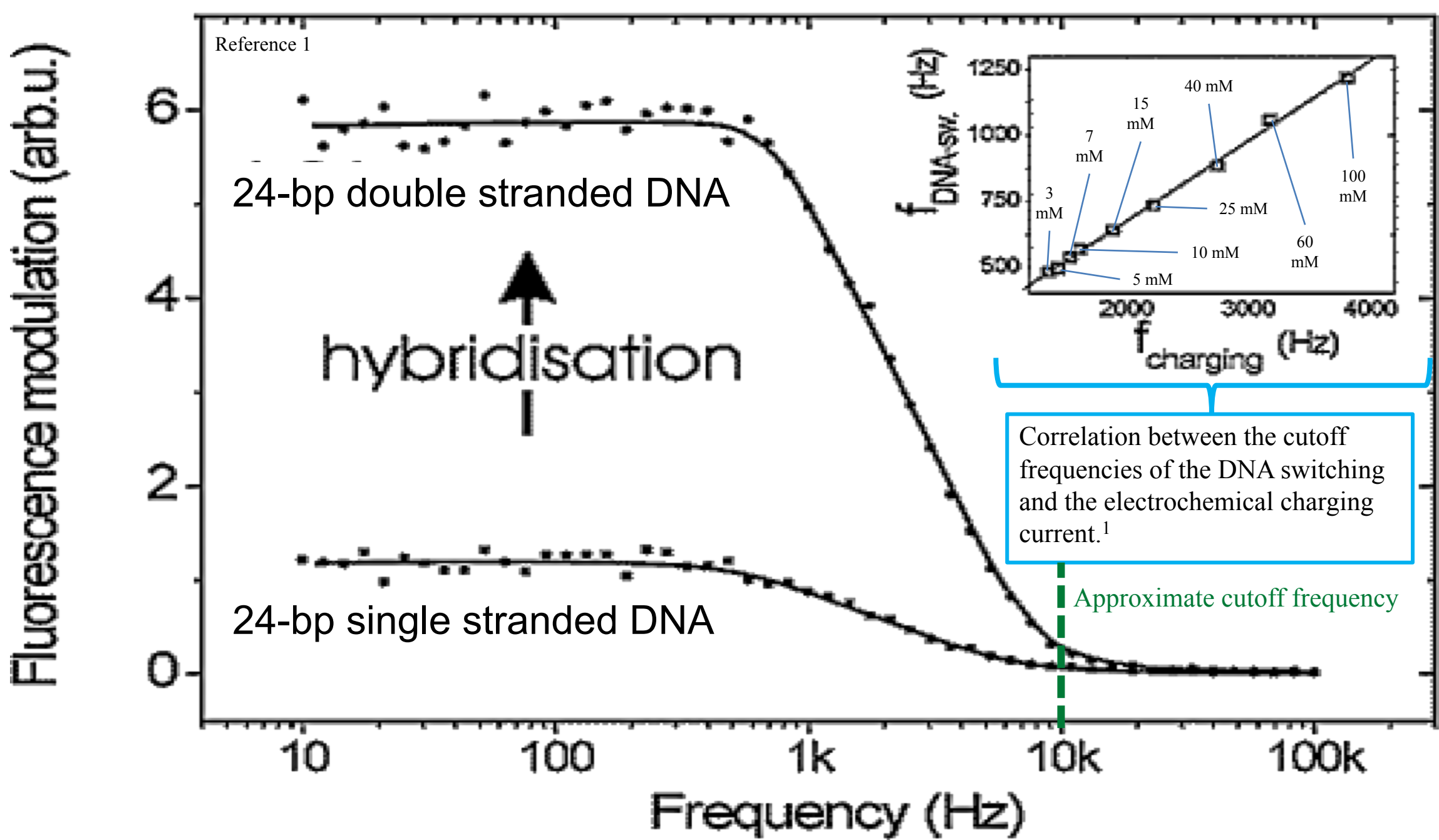
- Double stranded DNA (dsDNA) is made up of two anti-parallel polymer chains bound in a helix via non-covalent interactions, specifically pi-pi stacking and hydrogen bonds.
- Each chain consists of a sequence of nucleobases along a **negatively charged sugar-phosphate backbone**
- Electrostatic melting is the unzipping of (surface-tethered) dsDNA due to electrostatic repulsion.
- The electrostatic mechanism: The negatively charge backbone is repulsed by the negatively charged electrode charge, causing the dsDNA to unzip.
- This “electrostatic mechanism” is commonly assumed but not proven.
- Furthermore, there is some evidence that this mechanism is not correct.

## Time-scale of DNA Response

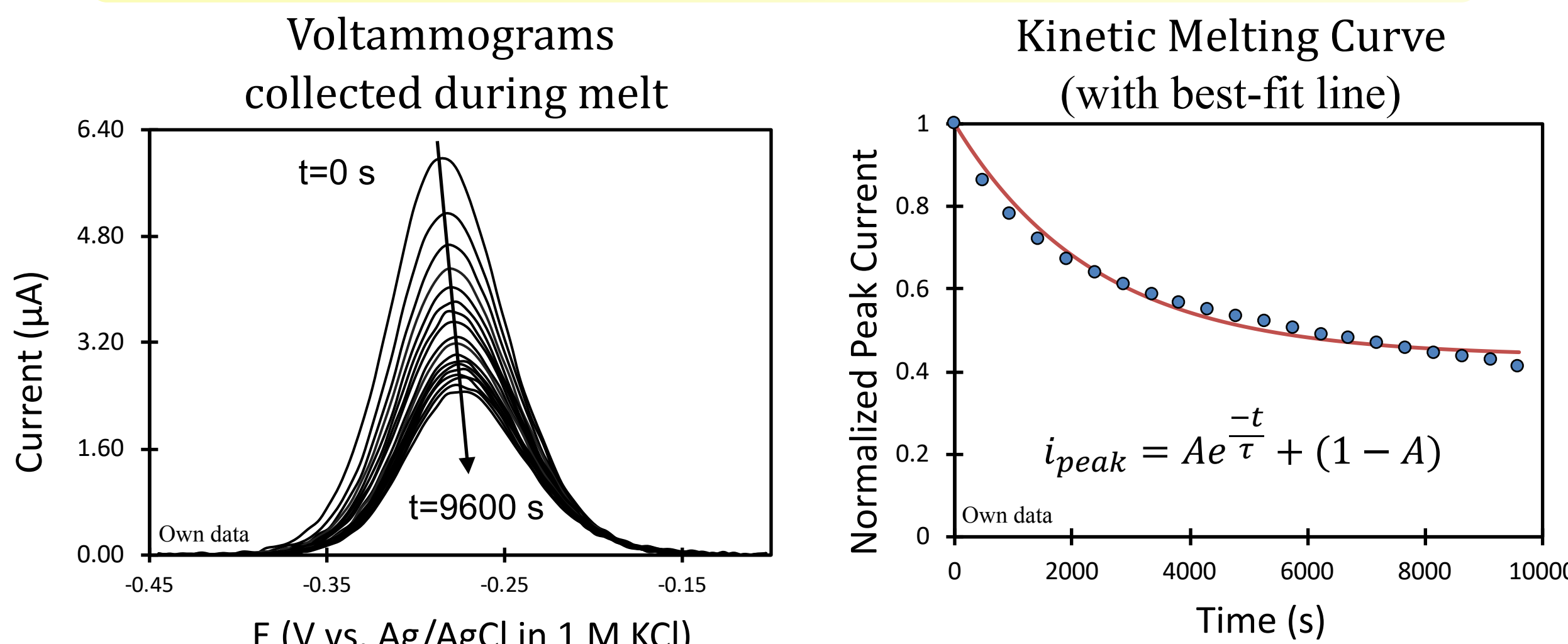
- Rant et. al. alternated between an attractive and a repulsive potential in order to influence the orientation of electrode-bound DNA.
- Rant et. al only intended to control the orientation of the DNA and thus used potentials that were too weak to cause melting.<sup>1</sup>



- The shorter the time period between the two potentials, the faster the DNA moves, until it is no longer able to respond (approximately 10 kHz – as seen below).



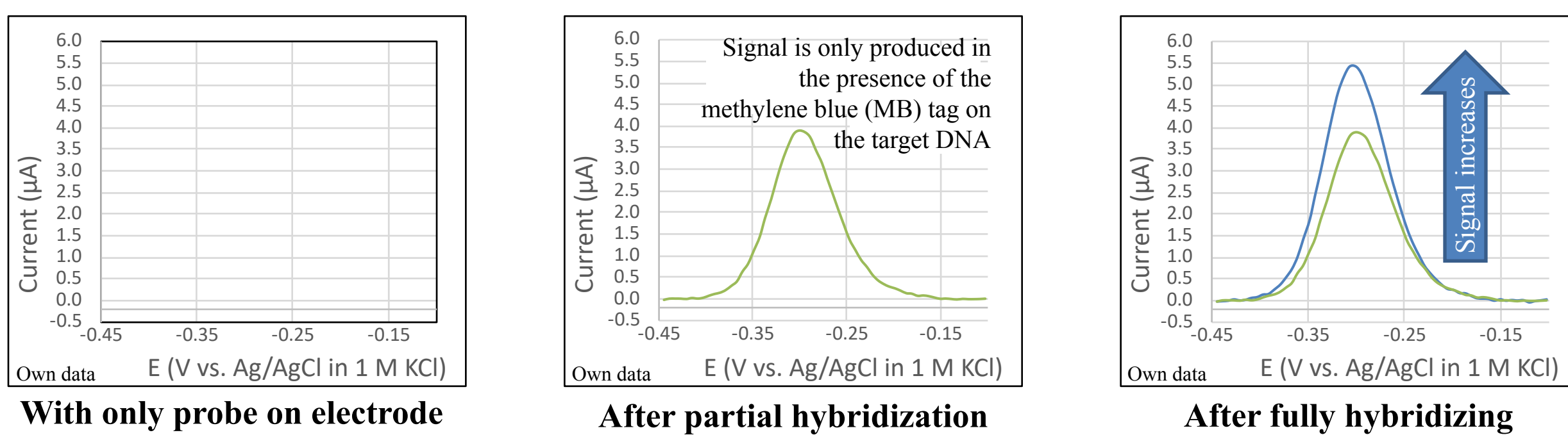
## Melting curves and data analysis



- The voltammograms (left) show a cathodic peak due to the reduction of the MB on the target strands.
- After each step of the melt, this peak decreases due to loss of the target DNA during melting.
- The baseline-subtracted peak currents are plotted versus time (right)
- Each curve is fit to obtain:
- The time constant,  $\tau$ :** rate of melting
- The extent of melting,  $A$ :** fraction of target melted

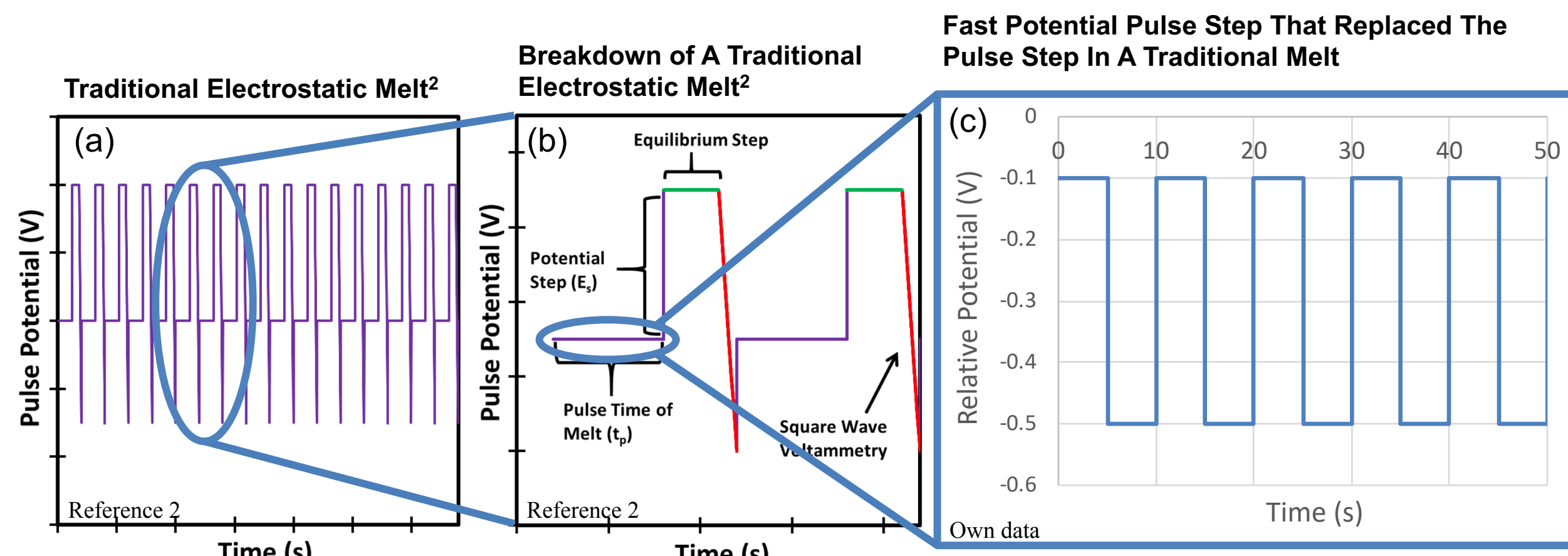
## Experimental

### Electrode preparation and e-melting



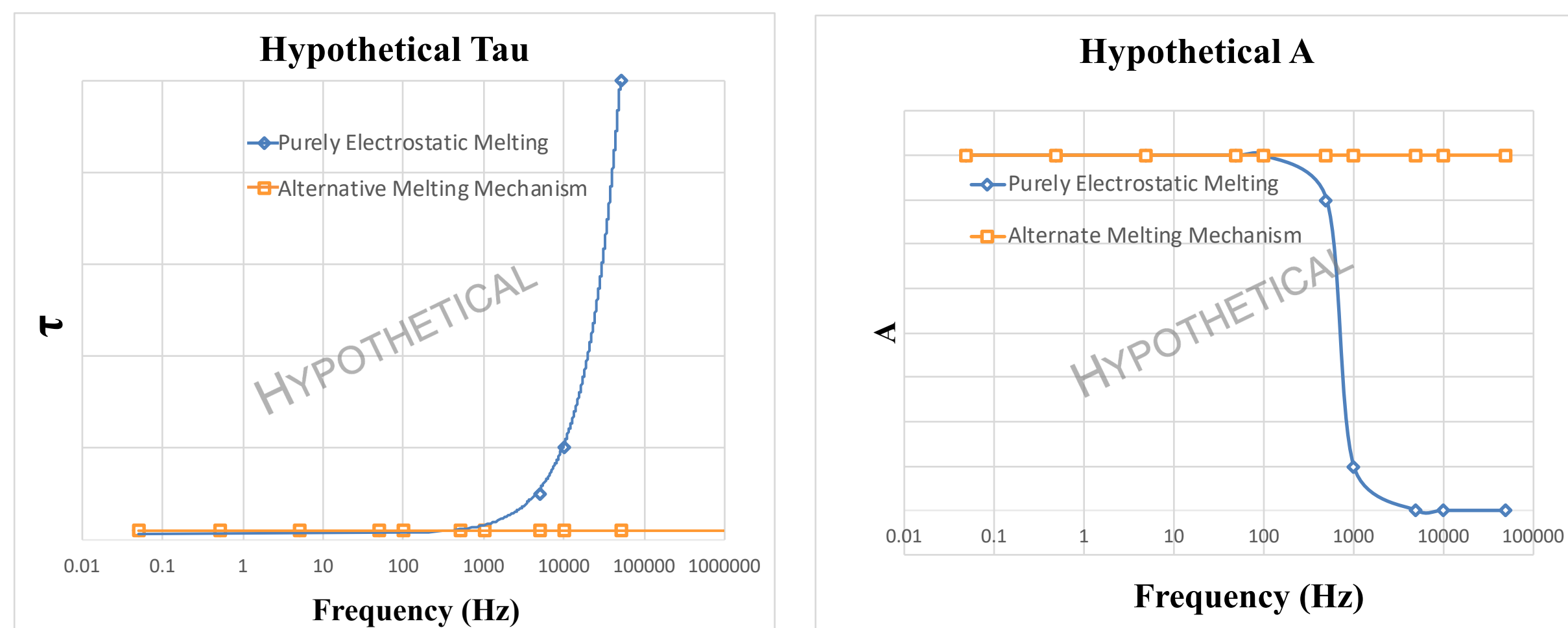
- Mixed monolayers of DNA and mercaptohexanol (MCH) are prepared via the backfill method, where probe is attached to the electrode via potential pulsing from -100 mV to -500 mV at 10 ms each for 15 minutes, followed by overnight incubation in MCH
- Modified electrodes are incubated in a solution of the target DNA at room temperature for 120 minutes. As shown in the above figure, the probe hybridizes with the tagged target DNA strand. Signal is produced by interactions between the methylene blue (MB) tag on the target strand and the electrode surface.
- Duplex modified gold electrodes are used as the working electrode in a 3-electrode cell. The electrolyte is 10 mM Tris buffer (pH 7)
- Electrochemical melting procedure (Figure 2): 8 min at alternating potentials of -500 mV and -100mV followed by 10 s equilibration step at -100 mV followed by SWV. Repeat.

18-bp Duplex (MB on target)	Probe: 5' HS-C6-TTG ATC GGC GTT TTA TTC 3' Target: 3' (MB) - AAC TAG CCG CAA AAT AAG 5'
-----------------------------	--

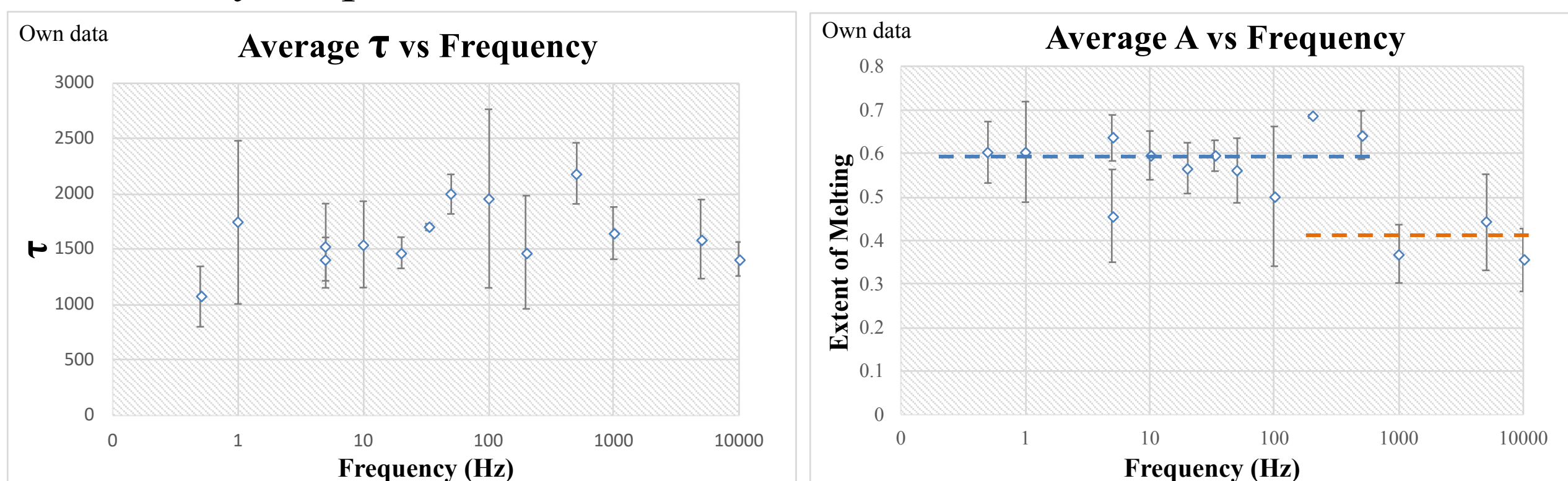


## Results and Discussion

### Comparison of Obtained Results to Hypothetical



- If electrostatic DNA melting has a purely electrostatic mechanism, the expected results of this experiment should show that after a certain frequency,  $\tau$  should effectively increase to infinity and  $A$  should essentially drop to zero.



- The results do not follow the theory of a purely electrostatic melting mechanism, as shown by the lack of significant change in  $\tau$ , regardless of frequency.
- The general trend of  $A$  seems to show that  $A$  is lower at higher frequencies.

## Conclusions

- Results indicate that electrostatic DNA melting does not have a purely electrostatic mechanism, but further research needs to be done in order to determine that the signal decay is solely due to DNA denaturation and not due to thiol bond reduction.
- Determination of the response time of the ions in the solution used is required.
- In future, a repeat of this experiment using an uncharged DNA mimic such as morpholino or PNA would help to verify the results found in this project.

## Acknowledgments

- We would like to acknowledge the USF Faculty Development Fund for financial support.

### References

- Ulrich Rant et al., Dynamic Electrical Switching of DNA Layers on a Metal Surface, *Nano Lett.*, **2004**, 4, 12, 2441-2445, <https://doi.org/10.1021/nl0484494>, Accessed 20<sup>th</sup> February 2020
- Ho, D.; Hetrick, W.; Le, N.; Chin, A.; West, R. M.; Electric Field-Induced DNA Melting with Detection by Square Wave Voltammetry, *Journal of The Electrochemical Society*, **2019**, 166(4), B236, <https://iopscience.iop.org/article/10.1149/2.0671904jes/meta> (Accessed on 16th February 2020)